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GAS CHROMATOGRAPHIC DETERMINATION OF MICROAMOUNTS OF CARBARYL AND 1-NAPHTHOL IN NATURAL WATER AS SOURCES OF WATER SUPPLIES

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SUMMARY

A method for the clean-up and quantitative determination of Carbaryl and its hydrolysis product, 1-naphthol, in natural waters is described. After extraction of a water sample with methylene chloride, the two compounds were separated from possible organochlorine pollutants such as Endrin, γ-BHC, *p,p'*-DDT, pentachlorophenol and polychlorinated biphenyl, and their heptafluorobutyryl derivatives obtained. Determination by electron-capture gas chromatography at the 2.5–10 ppb level, using 1 l of water samples, was carried out.

INTRODUCTION

Recently, the use of both organochlorine and organophosphorus pesticides has been severely controlled owing mainly to their residual toxicity, and the consumption of carbamate-type pesticides, which are relatively low in toxicity and short in their residual period, has increased. Carbaryl (N-methyl-1-naphthylcarbamate), above all, has been widely used for agricultural pest control, and possible pollution of sources of water supplies with Carbaryl is of increasing concern. Since Carbaryl is somewhat volatile and slightly soluble in water (40 ppm), it disappears fairly rapidly from the surface of farm products and its half-life has been reported as 3–4 days[1]. Disappearance of Carbaryl spread on soil or that penetrating into the soil with rain-water is also fast, with a reported half-life of *ca.* 7 days[2].

It has been reported that the decomposition of Carbaryl into 1-naphthol and N-methylcarbamyl moieties in water is affected by both pH and mud in the water, and the decomposition in soil is affected likewise by both pH and moisture[3,4]. Thus, evaluation of water pollution with Carbaryl necessitates observation of concentrations of both Carbaryl and its decomposition product, 1-naphthol, in natural waters such as the river, lake and well waters. There is an increasing need for an effective clean-up procedure and a more sensitive determination of Carbaryl and 1-naphthol. Various

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gas chromatographic (GC) procedures for the determination of residual carbamates in crops or environmental materials have been presented[5–9], most of which involve the derivatization of intact carbamate or of the phenol or amine resulting from its alkaline or acidic hydrolysis.

The work described here involves solvent extraction of Carbaryl and 1-naphthol from water samples, followed by partitioning to remove organochlorine pesticides and polychlorobiphenyl, and a GC determination of heptafluorobutyryl derivatives of Carbaryl and 1-naphthol.

EXPERIMENTAL

Reagents and pesticides

Methylene dichloride, hexane, and ether used were of analytical grade. Heptafluorobutyric anhydride (HFBA) for GC with an electron capture detector was obtained from Wakō (Tokyo, Japan). Pyridine of special reagent grade was dehydrated by distilling over barium oxide. 1-Naphthol of special reagent grade was recrystallized from aqueous ethanol to yield crystals of m.p. 94–95°, confirmed to be homogeneous by thin-layer chromatography (TLC). Other reagents and solvents used in this experiment were of special reagent grade.

Carbaryl of technical grade (Nissan, Tokyo, Japan) was recrystallized from aqueous ethanol to yield crystals of m.p. 139–140°, which gave a single spot on TLC. Hexachlorobenzene of special reagent grade was recrystallized from aqueous ethanol, and the 0.05 pppm solution in hexane gave no peak due to any impurities on its gas chromatogram. Pentachlorophenol (PCP) of special reagent grade was recrystallized from aqueous ethanol to yield crystals of m.p. 186–188°. Endrin, γ-BHC and *p,p'*-DDT, all analytical standard for GC, were used. Polychlorinated biphenyl (PCB, Kanechlor 400) was provided by Kanegafuchi (Tokyo, Japan).

A column (30 × 3 cm I.D.) packed with Amberlite XAD-8 resin (60 g, Rohm & Haas, Philadelphia, Pa., U.S.A.) was washed successively with 500 ml each of hexane, ether, ethanol, and ether, the resin was dried in air, and then heated at 60° overnight.

Preparation of standard solutions

A standard Carbaryl–naphthol solution in ethyl acetate was prepared, containing 10 μg/ml of Carbaryl and 10 μg/ml of 1-naphthol. The internal standard solution for GC analysis was hexane–ether (45:3) containing 0.05 ppm of hexachlorobenzene.

Apparatus

The gas chromatograph was a Shimadzu GC-4BM, equipped with a ^{63}Ni electron capture detector and a 200 cm × 3 mm I.D. U-shaped glass column. The operating conditions were: column temperature, 110–170° (3°/min); detector, 200°; nitrogen flow-rate, 40 ml/min; sensitivity, 10^2 *M*, 16 × 10^{-2} V.

Three GC columns were used. Column I, 2% Silicone XF-1105 on 60–80 mesh Gas-Chrom P; column II, 2% Silicone OV-17 on 60–80 mesh Chromosorb W AW DMCS; column III, 2% diethylene glycol succinate–0.5% H_3PO_4 on 60–80 mesh Chromosorb G AW DMCS.

Thin-layer chromatography

Samples were spotted on a glass plate (20 × 10 cm) with Kieselgel GF_{254} (0.25 mm thick), and developed by the ascending technique using the solvent system ethyl acetate–hexane (3:7). The spots of Carbaryl and 1-naphthol were detected by spraying *p*-nitrobenzenediazonium fluoroborate–KOH reagent[10], and 4-nitroso-1-naphthol was detected by its yellow coloration or by UV irradiation.

Colorimetric determination of Carbaryl and 1-naphthol

When monitoring of the solvent extraction of Carbaryl and 1-naphthol, or their derivatization for GC analysis was necessary, their colorimetric determination was carried out by the following methods[2,11]. After hydrolysis of Carbaryl with 0.2 *N* methanolic KOH, the product was coloured with a 0.03% solution of *p*-nitrobenzenediazonium fluoroborate in methanol, and its absorbancy at 590 nm (alkaline) was measured. 1-Naphthol was treated directly with a 0.1% solution of *p*-nitrobenzenediazonium fluoroborate in methanol, and its absorbancy at 470 nm (neutral) was measured.

Extraction and concentration

Acetate buffer (5 *M*, pH 4, 10 ml) and sodium chloride (30 g) were successively added to the water sample (1 l), and the sample solution was extracted in a 2-l separatory funnel with 100 ml of methylene chloride by shaking for 2–3 min. The methylene chloride extract was transferred to a 300-ml conical flask and the aqueous layer was re-extracted with another 100 ml of methylene chloride. The methylene chloride extracts were combined and passed through a column packed with anhydrous sodium sulphate (*ca.* 50 g) into a round-bottomed flask with suction. Both the conical flask and column were rinsed with 20–30 ml of methylene chloride. The methylene chloride extract and washings were combined and evaporated to *ca.* 0.5 ml in a Büchi-type rotary evaporator below 30° under reduced pressure with an aspirator. The concentrate was dried by gently passing nitrogen gas through a capillary, and was redissolved in 2–3 ml of hexane.

Separation on Amberlite XAD-8 column

A slurry of Amberlite XAD-8 in hexane was introduced into a glass column (30 × 0.8 cm I.D.) with the aid of hexane to form a 13-cm resin bed, and a 2-cm layer of anhydrous sodium sulphate was placed on the top of the column. The hexane solution obtained as described above was placed in the column, and the column was eluted successively with 100 ml of ether–hexane (5:95) (fraction 1) and 50 ml of acetone–hexane (20:80) (fraction 2) at a flow-rate of 1 ml/min. Fraction 1 was discarded, and fraction 2 was evaporated to *ca.* 0.5 ml below 30° under reduced pressure. The concentrate was dried by gently passing nitrogen gas, and was dissolved in 1 ml of ethyl acetate.

Preparation of test solutions for GC analysis

An aliquot (0.2 ml) of the ethyl acetate solution obtained as described above was transferred to a 10-ml graduated test-tube (10 × 1.5 cm I.D.), and mixed with 0.01 ml of pyridine and 0.2 ml of HFBA. The test-tube was firmly sealed with a glass stopper, and left to stand at 30° for 60 min. After the reaction period, 4.5 ml of the

internal standard solution for GC analysis was added, the mixture was washed successively once with water (3 ml), twice with 0.05 *M* sodium hydrogen carbonate (3 ml), and once again with water (3 ml), by stirring on a Vortex mixer for 1 min each. The separated organic solvent layer was made up to 5 ml by the addition of hexane–ether (45:3), and then dehydrated by the addition of anhydrous sodium sulphate (2 g).

For preparing calibration curves, a series of test-tubes (10 × 1.5 cm I.D.) each containing 0.05, 0.1, 0.15, and 0.2 ml of Carbaryl–1-naphthol standard solution was prepared, and volumes were made up to 0.2 ml by the addition of ethyl acetate. The contents of each test-tube were treated with HFBA in the presence of pyridine, and the subsequent treatment was carried out as described above.

Gas chromatographic determination

The test solution prepared by the procedure described in the preceding section was analysed on column I under the GC conditions described above. The injection volume was 5 μl.

RESULTS AND DISCUSSION

Extraction and subsequent concentration of Carbaryl and 1-naphthol from water samples

Extraction of distilled water samples (1 l) spiked with 0.03 ppm Carbaryl was examined using hexane and methylene chloride as extracting solvents, two extractions with 100 ml of one of the solvents, and colorimetric determination. The average recovery with methylene chloride was 98%, which was higher than that with hexane (40%).

A distilled water sample (1 l) spiked with 0.03 ppm each of Carbaryl and 1-naphthol was extracted with methylene chloride, and average recoveries of 98 and 85%, respectively, were obtained. A distilled water sample containing 0.5 *M* sodium chloride and extracted at pH 2 with the same solvent, resulted in recoveries of 98.5 and 97% of Carbaryl and 1-naphthol, respectively. However, if nitrous acid is present in the sample, 1-naphthol is converted under these latter extraction conditions into 4-nitroso-1-naphthol, which is strongly absorbed on Amberlite XAD-8 resin, making impossible the determination of 1-naphthol. Re-examination of the extraction conditions showed that 4-nitroso-1-naphthol does not form above pH 4. Carbaryl and 1-naphthol were extracted with recoveries of 98.5 and 98% with methylene chloride from water samples to which a one hundredth volume of 5 *M* acetate buffer (pH 4.0) and sodium chloride had been added. The procedure described in Experimental was eventually adopted.

Apparatus and conditions for a rapid evaporation of the solvent in the presence of Carbaryl and 1-naphthol, both of which are somewhat volatile, were examined, and the use of a Büchi-type rotary evaporator below 30° was preferable for both good recovery and saving time.

Separation of organochlorine compounds by column chromatography

It can be assumed that most sources of water supply have been possibly polluted more or less with organochlorine pollutants which interfere in the determination of Carbaryl and 1-naphthol by a gas chromatograph equipped with an electron

capture detector. For the removal of organochlorine compounds such as Endrin, γ-BHC, *p,p'*-DDT, PCP and PCB by column chromatography, Amberlite XAD-8 resin was selected as adsorbent after comparative experiments on several materials. Hexane solutions (1 ml each) containing the indicated amounts of Carbaryl, 1-naphthol, or one of the organochlorine compounds mentioned above were separately placed on seven columns of Amberlite XAD-8 resin prepared as described in the legend to Fig. 1. Each column was successively eluted with ether–hexane (5:95), acetone–hexane (20:80), and acetone alone, and the elution patterns of Fig. 1 were obtained. As can be seen, both Carbaryl and 1-naphthol were completely separated from all the organochlorine compounds except γ-BHC, which did not interfere with the GC–electron capture detection analysis of HFB derivatives of Carbaryl and 1-naphthol. Based on these experiments, a separation procedure for both Carbaryl and 1-naphthol from the organochlorine compounds was established, as described above *(Separation on Amberlite XAD-8 column)*. When the amount of Carbaryl and 1-naphthol in the concentrate separated from the organochlorine compounds is 2.5–10 μg, the use of 1 ml of ethyl acetate is adequate for their dissolution.

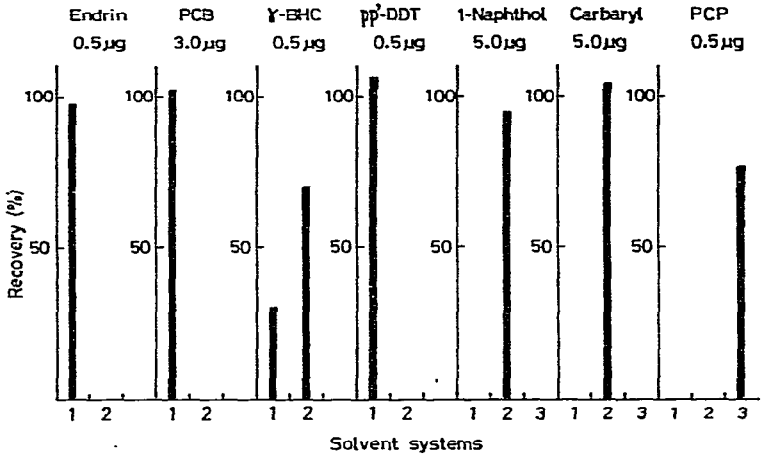

Fig. 1. Elution diagrams of Carbaryl, 1-naphthol, and organochlorine compounds on Amberlite XAD-8 columns. Hexane solutions (1 ml each) containing the indicated amount of one of the compounds were separately loaded on the column (30 × 0.8 cm I.D.), and each column was successively eluted with the following solvent systems: 1, ether–hexane (5:95, 100 ml); 2, acetone–hexane (20:80, 50 ml); and 3, acetone (50 ml). Recoveries of the organochlorine compounds were determined by GC analysis on column III, and those of Carbaryl and 1-naphthol were analysed on column I after conversion into their HFB derivatives.

Derivatization of Carbaryl and 1-naphthol to their heptafluorobutyryl compounds

Since the superiority of ethyl acetate as a solvent in the trifluoroacetylation or heptafluorobutyrylation of N-methylcarbamates has been reported[8], and since it is also known that a minute amount of pyridine accelerates the N-acylation[11], a solution of Carbaryl in ethyl acetate was treated with HFBA in the presence of pyridine at 30°. As shown in Fig. 2, the reaction mixture containing 0.01 ml of pyridine was better for the formation of heptafluorobutyryl(HFB)-Carbaryl. Application of this method to 1-naphthol also gave good results. The procedure established for HFB derivatiza-

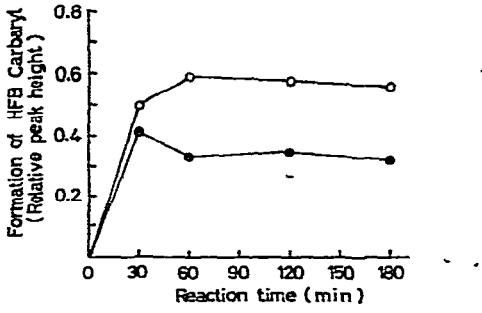

Fig. 2. Time courses of the formation of N-heptafluorobutyryl-Carbaryl. ○, 0.01 ml pyridine; ●, 0.1 ml pyridine. Carbaryl (1.0 μg) in ethyl acetate (0.2 ml) was treated with HFBA (0.2 ml) in the presence of the indicated amount of pyridine at 30°. The reaction mixture was treated as described in the Experimental section, and the formation of HFB derivative of Carbaryl was analysed by GC using column II.

tion of Carbaryl and 1-naphthol is detailed in the Experimental section. Calibration graphs for HFB-Carbaryl and HFB-1-naphthol in the range 2.5–10 μg per l of water sample were prepared, and a good linearity was obtained for both compounds, as shown in Fig. 3. Peak response of HFB-1-naphthol was *ca.* 3.5 times that of HFB-Carbaryl.

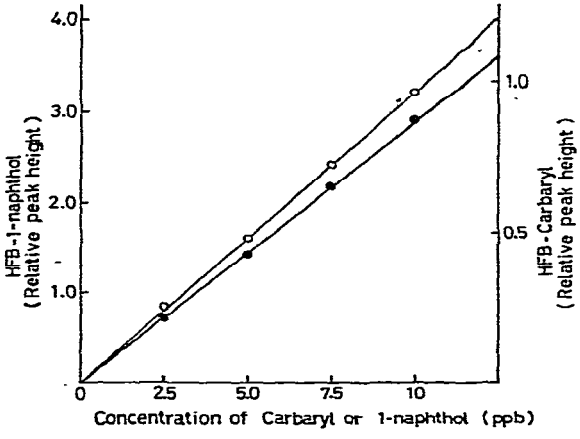

Fig. 3. Calibration graphs for Carbaryl and 1-naphthol in the concentration range 2.5–10 μg per l of distilled water. Operating conditions: Column I, 2% Silicone XF-1105 on 60–80 mesh Gas-Chrom P; column temperature, 110–170° (3°/min); electron capture detector, 200°; nitrogen flow-rate, 40 ml/min; volume injected, 5 μl. ○, 1-Naphthol; ●, Carbaryl.

A gas chromatogram of HFB derivatives of Carbaryl and 1-naphthol is shown in Fig. 4, and the peaks are better in their relative positions, retention times, and peak responses than those of corresponding trifluoroacetyl derivatives. A small peak with a retention time of 10 min was considered to be an unknown product originating from HFBA during the reaction, judging from the results of an experiment performed without addition of Carbaryl and 1-naphthol.

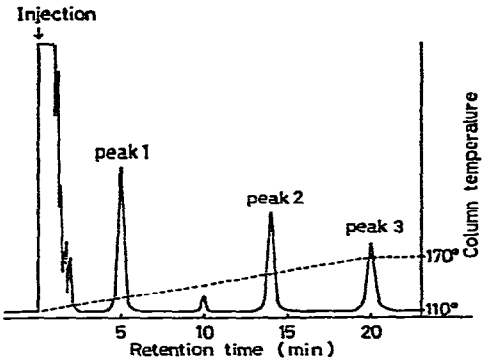

Fig. 4. Gas chromatogram of heptafluorobutyryl derivatives of Carbaryl and 1-naphthol. Operating conditions are the same as those described in the legend of Fig. 3. Peak 1, HFB-1-naphthol; peak 2, hexachlorobenzene (internal standard); peak 3, HFB-Carbaryl.

Recovery test of Carbaryl and 1-naphthol

A methanol solution (1 ml) containing Carbaryl (5 μg) and 1-naphthol (5 μg) was added to 1 l each of distilled water, a river water sample, and a well water sample. These natural water samples, collected from the lower reaches of the Tama river in the suburbs of Tokyo and from the agricultural district in Saitama prefecture, respectively, proved to be not polluted with Carbaryl or 1-naphthol when the present method was applied, but the river water was polluted with 0.24 ppm of nitrous acid. The recoveries of Carbaryl and 1-naphthol added to these water samples were determined by the present method and good results obtained, as shown in Table I.

TABLE I

RECOVERY OF CARBARYL AND 1-NAPHTHOL FROM DISTILLED WATER AND NATURAL WATER SAMPLES

Water sample		*Amount added (μg/l)*	*Recovery (%)*
Distilled water	Carbaryl	5	102
	1-Naphthol	5	90
River water*	Carbaryl	—	N.D.***
		5	97
	1-Naphthol	—	N.D.
		5	86
Well water**	Carbaryl	—	N.D.
		5	94
	1-Naphthol	—	N.D.
		5	82

* Collected from the lower reaches of the Tama river in the suburbs of Tokyo.
** Collected from the agricultural district in Saitama prefecture, Japan.
*** N.D. = not detected.

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